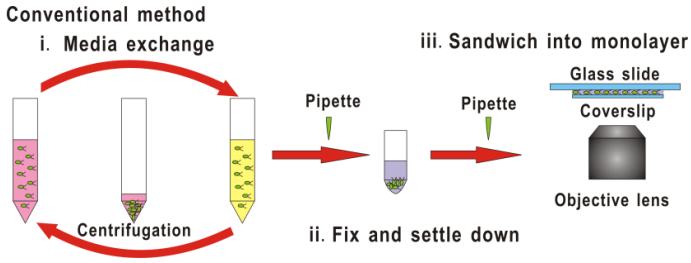
Supplementary Information

The conventional method of deflagellation, regeneration and flagellar length measurement

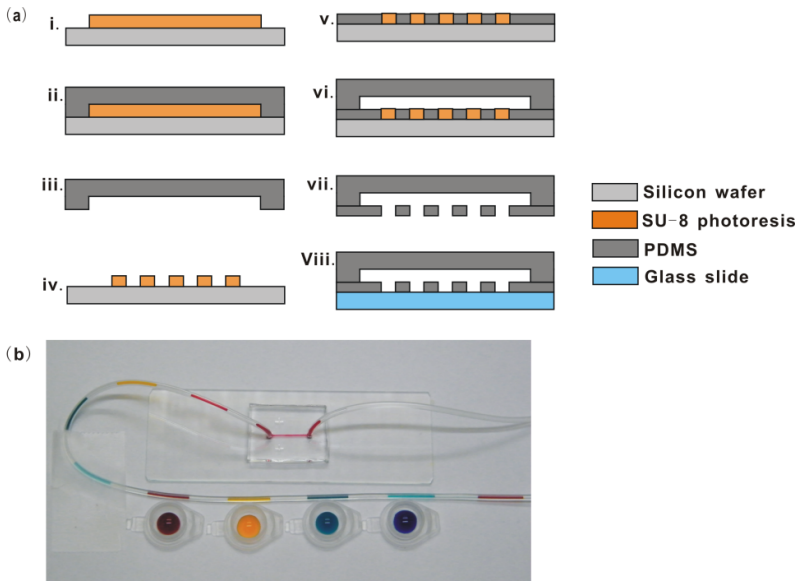
Deflagellation and flagellar assembly was essentially as described by Pan *et al*. The pH of *Chlamydomonas* cell culture was rapidly decreased to 4.5 by addition of 0.5 M acetic acid. After 30 s for flagella detachment, the pH was raised to 7.2~7.4 with a certain volume of 0.5 M KOH. After centrifugation, the sedimented cells were resuspended in a fresh M medium for flagellar regeneration. 200 µl cell samples for flagellar length measurements were fixed in a final 1% glutaraldehyde solution at different stages and imaged using an inverted Olympus IX71 microscope with a 60× objective (with 1.6× magnification changer). Flagellar length results were graphed using GraphPad Prism version 5.0a.

M medium

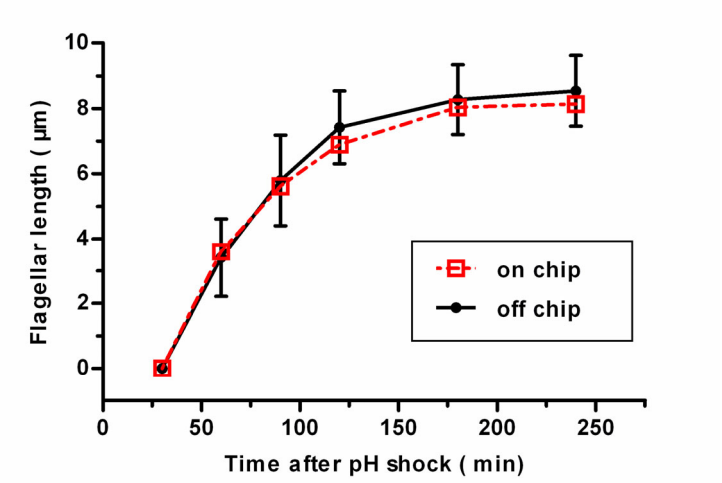
Sodium Citrate (0.5 g), Ferric Chloride (0.03 g), Calcium Chloride (0.053 g), Magnesium Sulfate (0.3 g), Ammonium Nitrate (0.3 g), Potassium Phosphate (0.1 g), Dipotassium Phosphate (0.131 g), H_3BO_3 (0.001 g), $ZnSO_4 \cdot 7H_2O$ (0.001 g), $MnSO_4 \cdot H_2O$ (0.000303 g), $CoCl_2 \cdot 6H_2O$ (0.0002 g), $Na_2MoO_4 \cdot 2H_2O$ (0.0002 g), $CuSO_4 \cdot 5H_2O$ (0.0000625 g), add DI water to 1L.



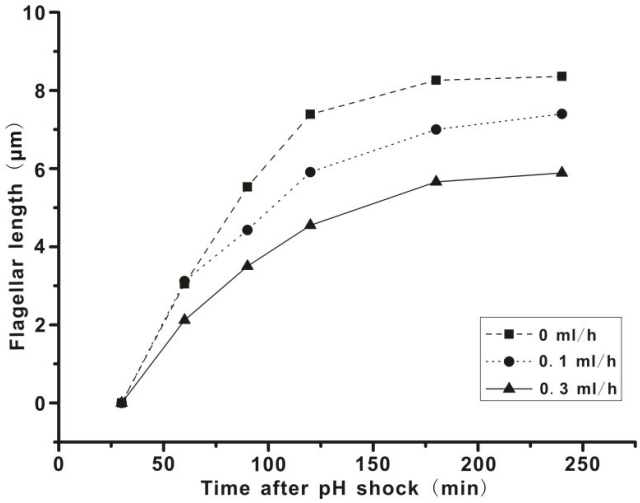
Supplementary Fig. 1 Schematic illustration of the conventional method for analysis of *Chlamydomonas* flagella. This includes the following artificial steps: i) Exchange media by centrifugation for 3 min and circulate the process if washing is necessary. ii) Sampling by pipettting at certain intervals, and cell fixation. iii) Sandwich cells between a glass slide and a coverslip into monolayer for imaging under a microscope.



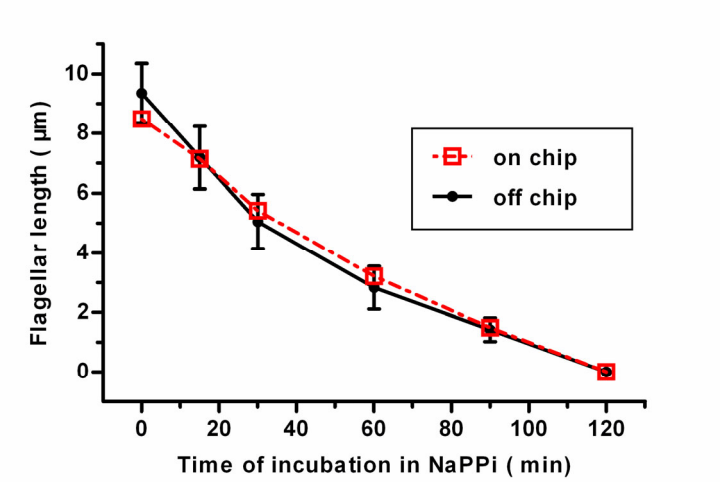
Supplementary Fig. 2 (a) Flow diagram of fabrication procedure of the microfluidic device. (b) The photograph below showing the microdevice with pre-loading sequential gas-liquid plugs into a tubing for media isolation. The liquid plugs are filled with food dyes.



Supplementary Fig. 3 On-chip and off-chip comparisons of flagellar regeneration after pH shock. At least 50 cells were measured for each time point. Red dashed line with square symbols presents results from the on-chip method. Black solid line with round symbols presents results from the traditional method, and error bars indicate SD. Flow rate: 0 ml/h.



Supplementary Fig. 4 On-chip flagellar regeneration *versus* flow rate. At least 50 cells were measured for each time point. Square symbols present static flow (0 ml/h), round symbols present 0.1 ml/h, and triangle symbols present 0.3 ml/h.



Supplementary Fig. 5 On-chip and off-chip comparisons of flagellar disassembly during incubation in 20 mM NaPPi for 120 min. Red dashed line with square symbols presents results from the on-chip method. Black solid line with round symbols presents results from the traditional method, and error bars indicate SD. Flow rate: 0 ml/h.

Supplemental References

1. J. M. Pan and W. J. Snell, J. Biol. Chem., 2000, 275, 24106-24114.